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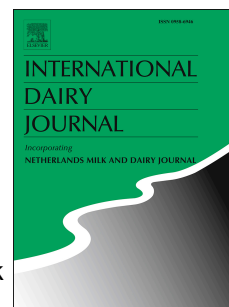
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**Pilot-scale ceramic membrane filtration of skim milk for the production of a
protein base ingredient for use in infant milk formula**

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ABSTRACT

The protein composition of bovine skim milk was modified using pilot scale membrane filtration to produce a whey protein-dominant ingredient with a casein profile closer to human milk. Bovine skim milk was processed at low (8.9 °C) or high (50 °C) temperature using ceramic microfiltration (MF) membranes (0.1 µm mean pore diameter). The resulting permeate stream was concentrated using polyethersulfone ultrafiltration (UF) membranes (10 kDa cut-off). The protein profile of MF and UF retentate streams were determined using reversed phase-high performance liquid chromatography and polyacrylamide gel electrophoresis. Permeate from the cold MF process (8.9 °C) had a casein:whey protein ratio of ~35:65 with no α_s - or κ -casein present, compared with a casein:whey protein ratio of ~10:90 at 50 °C. This study has demonstrated the application of cold membrane filtration (8.9 °C) at pilot scale to produce a dairy ingredient with a protein profile closer to that of human milk.

1. Introduction

The design of membrane-based fractionation processes for the development of enriched protein ingredients can provide an enhanced nutritional profile in infant milk formulations (IMF). The compositional differences between human and bovine milk are of particular importance for infant food formulations as the majority of infant formula is based on bovine milk (O'Mahony & Fox, 2013). The use of unmodified bovine milk as the sole source of nutrients for infants is discouraged (Morgan, 2006). Most current-generation IMFs consist of milk-derived ingredients, vegetable oils and micronutrients blended together in proportions consistent with those found in human milk (Martin, Ling, & Blackburn, 2016). The protein component of IMFs is critical to support the growth and development of infants, but achieving the correct protein profile in IMFs is complicated by milk protein heterogeneity and inter-species variation.

As described by O'Mahony and Fox (2013), milk proteins can be divided into two principal families, caseins (CNs) and whey proteins (WPs), which can be further sub-divided into individual CNs (e.g., α_S -CN, β -CN, κ -CN) and WPs (e.g., α -lactalbumin, β -lactoglobulin, lactoferrin). The protein profile of IMFs intended for infants between 0–6 months ('first age') is designed to closely mimic that found in human milk. WPs account for ~60% of the total protein present in human milk, compared with only ~20% in bovine milk (de Wit, 1998); thus, IMFs are typically prepared by adjusting the CN:WP ratio of bovine milk (80:20) to that of human milk (40:60) by mixing skim milk with WP-based ingredients (e.g., whey protein concentrate and demineralised whey). The CN and WP fractions of human milk consist primarily of β -CN and α -lactalbumin, respectively, while α_S -CNs and β -

lactoglobulin predominate in bovine milk (Lönnerdal, 2003). Increasing the proportion of proteins such as β -CN and α -lactalbumin is an important objective in efforts to improve the nutritional properties of IMFs and can be achieved through the addition of enriched or purified protein ingredients (Fox, Uniacke-Lowe, McSweeney & O'Mahony, 2015).

The need to use multiple dairy ingredients to achieve a humanised protein profile places additional burdens on IMF manufacturers in terms of ingredient sourcing/transit and prediction of ingredient functionality/stability during reconstitution, thermal processing and re-drying. An alternative strategy is here proposed where the development of 'protein base' ingredients, in which several of the major humanisation targets (CN:WP ratio, CN profile) are achieved in a single processing operation. Such a strategy is feasible using a process based on small-pore (0.1 μ m) microfiltration (MF), described in the present study.

Although there have been major developments in the manufacture of WP-based ingredients for IMFs (e.g., enriched α -lactalbumin fractions, purified lactoferrin), progress in CN ingredient development has been comparatively slow. 'Cold MF' (i.e., filtration temperature: 0 to 15 °C) of skim milk is a promising approach to generating β -CN-enriched WP-dominant ingredients for use in the production of IMFs. There are a number of studies that have demonstrated the enrichment of β -CN using MF (or sometimes UF) at temperatures < 10 °C (Crowley et al., 2015; Le Berre & Daufin, 1994; O'Mahony, Smith, & Lucey, 2014; Woychik, 1992). Crowley et al. (2015) recently demonstrated the concept at laboratory scale using polyvinylidene-difluoride (PVDF, 0.1 μ m pore size) or polyethersulfone (PES, 1000 kDa cut-off) membranes at temperatures <5 °C. It is possible to effectively enrich β -CN from renneted milk gels (Huppertz et al., 2006), but MF has the

advantage of generating a soluble and highly-functional co-product, micellar casein concentrate (MCC). The enrichment of β -CN in the whey permeate during cold MF is based on the principle that an increasing proportion of β -CN is monomeric as temperature is decreased, with a concomitant migration of the monomers from micelles into the serum phase (Dauphas et al., 2005; Rose, 1968). β -CN can be manufactured at relatively high purity (70–80% of total protein) by cold MF of liquid MCC (Christensen & Holst, 2014) or warm MF (>26 °C) of the β -casein-enriched whey permeate generated from cold MF of milk; however, for the production of WP-dominant IMFs pure β -casein is not an essential requirement, as the presence of WPs in the ingredient is likely preferable.

Most of the MF processes that have been described for β -CN enrichment have used cold temperatures and polymeric membranes, and have been associated with low permeate flux values, due to a combination of high feed viscosity and severe fouling of the membrane. Strategies to improve flux are primarily restricted to adjusting the type of membrane; for example, it has been shown that flux-enhancement in a cold MF process can be achieved by using PES rather than PVDF membranes (Crowley et al., 2015). However, it can be predicted that a switch to ceramic membranes would facilitate operation at much higher flux values. While the surface area of ceramic membrane configurations may not be as large as for spiral-wound polymeric systems, they have the advantage of being compatible with technology designed to ensure a consistent trans-membrane pressure (TMP) across the length of the membrane. An example of this technology is the Isoflux® membrane, in which the active layer decreases in thickness from inlet to outlet, so that the pressure:thickness ratio, and therefore flux, is theoretically constant along the hydraulic path (Adams & Barbano, 2013).

The objective of this study was to produce an ingredient from skim milk for application as a protein base during IMF manufacture. The target protein profile for the ingredient was a CN:WP ratio close to that of human milk, in which the CN fraction consisted primarily of β -CN. The ingredient was prepared using MF and DF of skim milk at low temperatures using ceramic Isoflux[®] membranes, followed by concentration using UF. A cold MF process was carried out and compared with the more traditional warm MF in terms of process performance (i.e., permeate flux) and nutrient partitioning (i.e., minerals, proteins, non-protein nitrogen and fat).

2. Materials and methods

2.1. Materials

Raw bovine whole milk was obtained from the Teagasc Grassland Research Centre, with the fat separated centrifugally at 50 °C using facilities at Moorepark Technology Limited (Fermoy, Co. Cork, Ireland). Urea, bis-tris propane, and 2-mercaptoethanol were obtained from Sigma (Wicklow, Ireland). The electrophoresis chemicals were obtained from Bio-Rad (Fannin, Dublin, Ireland). All other chemicals used were of analytical grade. Milli-Q water (Millipore, Ireland) was used for all solutions.

2.2. Compositional analysis

Total nitrogen, non-protein nitrogen and true protein were determined using the Kjeldahl method (ISO, 2001a,b,c), and a nitrogen-protein conversion factor of

6.38. Fat content was determined using the Gerber method (IDF, 1991). Mineral analysis was determined by an Agilent 7700s inductively-coupled plasma mass spectrometry (ICP-MS) (Agilent Technologies, Santa Clara, California, USA).

2.3. Pilot-scale membrane filtration and powder manufacture

Skim milk (300 kg) was diluted prior to filtration by adding 600 kg of reverse osmosis (RO) water. In-house testing has shown that diluting unheated skim milk prior to filtration increased the dissociation and solubilisation of β -casein. The diluted skim milk was held at ~ 4 °C for 16 h, before holding at 8.9 °C or heating to 50 °C prior to membrane filtration. The diluted skim milk was subject to MF using 0.14 μm pore size Tami Isoflux[®] ceramic membranes (Tami Industries, Nyons Cedex, France) on a GEA Model F filtration unit (GEA Process Engineering A/S, Skanderborg, Denmark) with complete retentate recycling (i.e., retentate is returned to the feed). Three ceramic membranes were used in parallel, each with an area of 0.35 m². The temperature throughout processing was maintained at 8.9 ± 1.2 °C or 50 ± 1.0 °C using an in-line heat exchanger. The feed recirculation rate was adjusted to 1500 L h⁻¹ at a feed pressure of 1 bar and a membrane inlet pressure of 2.7 bar (0.9 bar per membrane element). The permeate flux was measured gravimetrically throughout filtration until a volume concentration factor (VCF) of 3 was reached. VCF was calculated by dividing the feed volume (V_f) by the final retentate volume (V_r):

$$\text{Volume concentration factor } VCF = \frac{V_f}{V_r} \quad (1)$$

All permeate streams from MF were subsequently subjected to UF with two 10 kDa cut-off Synder spiral-wound membranes (Synder Filtration, CA, USA)

arranged in series in the Model F filtration unit. UF was performed at a temperature of 50 ± 1.0 °C for all trials with a feed recirculation rate of 1600 L h^{-1} at 1 bar pressure and a membrane inlet pressure of 1.8 bar. A final VCF of 14 was attained. Permeation behaviour of individual proteins through MF membranes were characterised using the sieving coefficient (S_o).

$$S_o = \frac{C_p}{C_R} \quad (2)$$

where C_p and C_R are the simultaneous concentration of the protein in the permeate and retentate, respectively.

Membrane filtration performance was monitored using permeate flux measurements taken during each MF concentration run (Fig. 1A); solids content of the feed was also monitored (Fig. 2B); the starting feed material was skim milk (9.3% solids) diluted to ~3.1% solids with RO water. The solids content and pH of the original skim milk and rehydrated powders (protein content 3.3%, w/w) were measured using a CEM Smart Trac moisture analyser (Damastown, Dublin, Ireland) and a WTW 3310 pH meter (WTW, Weilheim, Germany), respectively.

Retentate streams obtained from MF and UF were heated to 30 °C prior to evaporation using a Tetra Scheffers™ single-stage falling-film evaporator operated at 60 °C (Tetra Pak, Gorredijk, The Netherlands). The concentrate was spray-dried using a pilot scale Anhydro Lab 3 spray dryer (SPX Flow Technology A/S, Soeborg, Denmark), equipped with a wheel atomiser. Inlet and outlet temperatures were set at 178 °C and 88 °C, respectively. Powder samples were coded as follows: MFR_{warm} , dried retentate from MF of skim milk at 50 °C; MFR_{cold} , dried retentate from MF of skim milk at 8.9 °C; UFR_{warm} , dried retentate from UF of warm (50 °C) MF permeate; UFR_{cold} , dried retentate from UF of cold (8.9 °C) MF permeate.

Membrane filtration processes at 50 and 4 °C were carried out in triplicate from a total of six different batches of skim milk, producing three independent powder samples for each of MFR_{warm}, MFR_{cold}, UFR_{warm} and UFR_{cold}.

2.4. Protein profile analysis

Protein profile analysis of MF and UF retentate powder samples was carried out using reverse phase-high performance liquid chromatography (RP-HPLC) and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) to quantify individual protein fractions and to observe the presence of minor whey protein components, respectively.

2.4.1. Reversed phase-high performance liquid chromatography

All MF retentate and UF retentate powder samples were dispersed in buffer containing 7 M urea, 20 mM bis-tris propane and 71.5 mM 2-mercaptoethanol (pH 7.5) in a 1:20 ratio (v/v) at room temperature. Each sample was incubated for 1 h at room temperature before being filtered (pore size of 0.22 µm). A 5 µL aliquot of each sample was injected three times into a Poroshell 300SB-C18 (Size: 2.1 × 7.5 mm, 5 µm; Agilent Technologies, Ireland) column equipped with a Zorbax poroshell guard column (Size: 1.0 × 17 mm, 5 µm; Agilent Technologies). The HPLC was equipped with a UV-vis detector (61365D MWD Agilent Technologies 1200 series). The column was operated at temperature 35 °C at a flow rate of 0.5 mL min⁻¹ and was equilibrated in 74% solvent A (0.1% trifluoroacetic acid and 10% acetonitrile in MilliQ water) and 26% B (0.1% trifluoroacetic acid and 10% MilliQ water in acetonitrile). A series of linear gradients were then applied by raising the

concentration of solvent B to 37% over 10 min, then to 45% over 23 min, and to 100% over 3.5 min. Solvent B was held at 100% for 4.5 min before reducing its level over 34 min back to the initial level of 26% where it was held for 2.5 min. Detection was by absorbance at 214 nm and total run time was 36.5 min per sample. HPLC analysis was carried out in triplicate

2.4.2. *Sodium dodecyl sulphate-polyacrylamide gel electrophoresis*

SDS-PAGE analysis was performed based on the methods of Laemmli (1970) and modified as described by Oldfield, Singh, Taylor, and Pearce (2000). Reducing SDS-PAGE was run with the addition of 2-mercaptoethanol, with heating at 95 °C for 5 min. Gels were run on a Mini-Protean III dual cell system (Bio-Rad Laboratories, Hercules, CA, USA). Mini-Protean TGX gels (anyKda, 10 wells; Bio-Rad) were used in the analyses. MF retentate and UF retentate powder samples were diluted to 0.13% (w/w, protein basis) with the appropriate sample buffer before loading. Subsequently, the corresponding sub-samples were loaded accurately (10 µL per well) into the sample wells using a micro-pipette. Electrophoresis was carried out at 200 V. The bands were stained with 0.1% (w/v) Coomassie Brilliant Blue G250 in 34% methanol for 24 h and destained in a solution containing 10% (v/v) glacial acetic acid and 10% (v/v) isopropanol. The destaining solution was changed every 4 h to obtain a light background on the gel. Destained gels were scanned on Epson Scan Perfection V700 Photo scanner (Epson, Hertfordshire, UK) and the protein bands were identified using the software program, ImageQuant (GEHealth care, Ireland).

2.5. *Statistical analysis*

Fisher's one-way multiple-comparison test was used as a guide for pair comparisons of the mean permeate flux values and the powder compositional data produced from membrane filtration. The level of significance was determined at $P < 0.05$ using Minitab 17 (Minitab Ltd, Coventry, UK) statistical analysis package and the effects of treatment and replicates were estimated for each response variable.

3. Results and discussion

3.1. Membrane filtration process performance

The performance of the MF process under warm (50 °C) and cold (8.9 °C) conditions can be compared in Fig. 1A. Pre-dilution of the skim milk 16 h prior to MF was performed to promote β -CN dissociation. Low temperature increases serum-phase β -CN by reducing hydrophobic interactions, while both cold processing and milk dilution partially dissolve colloidal calcium phosphate (CCP), further increasing transmission of β -CN (Broyard & Gaucheron, 2015). Initial permeate flux (time 0) of $\sim 380 \text{ L m}^{-2} \text{ h}^{-1}$ for warm MF rapidly decreased to $\sim 307 \text{ L m}^{-2} \text{ h}^{-1}$ after 0.25 h; flux remained relatively constant at this value until $\sim 1.7 \text{ h}$ of processing and a solids content of $\sim 4.5\%$ when a sudden and dramatic decline in flux to $< 150 \text{ L m}^{-2} \text{ h}^{-1}$ occurred (Fig. 1A). Once the retentate reached $\sim 75\%$ protein (w/w, dry matter) the MF rig was shut down, coinciding with a total process time of $\sim 3 \text{ h}$ and a final solids content of 12.2% , w/w. Previous studies for the MF of skim milk at 50 °C have reported much lower permeate flux values than those measured in the current study; for example, Hurt, Zulewska, Newbold, and Barbano (2010) obtained a constant permeate flux for skim milk of $\sim 54.0 \text{ L m}^{-2} \text{ h}^{-1}$ using ceramic membranes with a pore-

size of 0.1 μm , operated under uniform trans-membrane pressure (0.42 bar) at 50 °C. The high flux rates shown in Fig. 1 are probably due to a combination of a high retentate flow rate (1500 L h⁻¹) and the use of highly dilute skim milk as the feed. MF carried out at 8.9 °C had a significantly ($P < 0.05$) lower initial permeate flux (~98 L m⁻² h⁻¹; Fig. 1A), compared with MF at 50 °C (~380 L m⁻² h⁻¹; Fig. 1A). Permeate flux continued to decrease in a linear manner throughout filtration (Fig. 1A), reaching a value of 52 L m⁻² h⁻¹ after ~13 h.

Although the average permeate flux was much lower for cold MF compared with the warm MF process, the extent of flux decline was much less for the former, due to reduced fouling (Fig. 1), in agreement with other research demonstrating the benefits of cold membrane processing. Luo, Ramchandran, and Vasiljevic (2015) compared the performance of UF membranes during processing of milk at 15, 30 or 50 °C and found a more rapid flux decline at the highest UF temperature due to increased protein- and calcium-based fouling. Similarly, Méthot-Hains et al. (2016) found that flux decline during UF of skim milk was more rapid at 50 °C than at 10 °C as the VCF increased from 1 to 4. The flux values shown in the present work for cold MF were significantly higher than values reported in previous studies for cold MF processes; although, diafiltration water was added upfront to the skim milk in the current study, which would significantly increase permeate flux. For example, O'Mahony et al. (2014) obtained an initial permeate flux of ~10 L m⁻² h⁻¹ during the enrichment of β -CN from skim milk using polymeric membranes at 3–6 °C.

3.2. Composition and physicochemical properties of dried retentates

The composition and physicochemical properties of MF and UF retentate powders after spray drying are shown in Table 1. Fat content increased in the MF retentates compared with the original skim milk, with very low levels of fat present in the UF retentates. There was a significantly ($P < 0.05$) lower fat content in UFR_{cold}, indicating that milk fat had lower transmission at the low temperature during cold MF.

The mineral composition of powders from MF and UF retentate streams are given in Table 3. Calcium and phosphorous levels decreased in MFR_{cold} compared with MFR_{warm}; in turn, UFR_{cold} had a higher calcium and phosphorous content compared with the UFR_{warm} powder. Similarly, Méthot-Hains et al. (2016) found that the permeation of calcium increased during UF of milk with 10 kDa PES spiral-wound membranes at 10 °C compared with 50 °C, due to an increase in soluble calcium at low temperatures. Magnesium, sodium and potassium were all significantly ($P < 0.05$) higher in UF retentates compared with MF retentate powders, while the temperature of MF processing had no significant effect ($P > 0.05$) on their transmission (Table 3). Therefore, MF temperature can be said to have only had an effect on the major components of CCP. The pH of MF and UF retentate streams were significantly ($P < 0.05$) higher than that of the diluted skim milk, likely due to the loss of these soluble minerals along with citric acid that would otherwise act as buffering salts (Table 1). Hurt et al. (2010) found a similar result during the MF of skim milk at 50 °C, when the pH of the retentate fraction increased from pH 6.6 to pH 7.0.

3.3. Protein profile of retentate streams from microfiltration and ultrafiltration

3.3.1. β -Casein transmission and casein:whey protein ratio

MF of skim milk at 50 or 8.9 °C resulted in very different protein profiles (Table 2). MF of skim milk at 50 °C facilitated extensive depletion of whey proteins with almost complete retention of casein (Fig. 2; lane 3 MFR_{warm}). UFR_{warm} powders contained primarily whey proteins (91.2% of protein) and a minor quantity of β -CN (8.81% of protein). SDS-PAGE profiles (Fig. 2; lane 1 UFR_{warm}) and HPLC chromatograms (Fig 3; profile E) indicated the presence of some β -CN in UFR_{warm} powders, quantified at 8.81% of total protein, with the remaining 91.2% consisting of WPs (Table 2). The average So of β -CN during MF at 50 °C was 0.07, which was significantly lower than the value of 0.26 measured during MF at 8.9 °C (Table 2; Fig. 3E).

Due to the high So values reached for β -CN and whey proteins during cold MF the UFR_{cold} powder had a CN:WP ratio of 35:65 with 100% of the CN comprised of β -CN (Table 1); this protein profile is close to that found in human milk and compares favourably in this respect with previous reports of MF processes involving similar pore-size membranes. For example, Woychik (1992) enriched β -CN using 0.1 μ m MF membranes at 4 °C; however, the β -CN purity was low (55% of total CN) and the system was CN-dominant (55:45 CN:WP). Glas, te Biesebeke, Kromkamp and Klarenbeek (2013) reported enriching β -CN from skim milk using MF with 0.15 μ m membranes at 10 °C, which resulted in a low CN:WP ratio of 14:86 and a high β -CN purity of 95% (total CN basis). Crowley et al. (2015) measured a β -CN purity of 100% and a CN:WP ratio of 49:51 when a permeate generated at <4 °C using a 0.1 μ m MF membrane was analysed. The process described in the present study achieves a balance of appropriate CN:WP ratio and excellent β -CN purity (confirmed using Maldi-TOF analysis; results not shown).

3.3.2. *Whey protein transmission and proteolysis products*

Cold MF resulted in greater retention of β -lactoglobulin (Fig. 2; lane 4), compared with warm MF (Fig. 2; lane 3). Gésan-Guiziou, Daufin, and Boyaval (2000) studied the factors affecting the transmission of β -lactoglobulin during MF of skim milk at 50 °C and showed that above the critical flux (i.e., pressure independent region) β -lactoglobulin transmission decreased significantly, due to modified fouling layer characteristics. The hindered protein transmission observed for cold MF in this study may also be caused by altered sieving characteristics due to an altered fouling layer; the increased hydrodynamic volume of the micellar phase at low temperatures (Liu, Weeks, Dunstan, & Martin, 2013) could be a possible explanation for this effect.

The detection of a band with a molecular mass of ~12 kDa in SDS-PAGE analysis of MF retentate streams may be the result of casein proteolysis occurring during membrane filtration (Fig. 2; lanes 3 and 4). Its absence from UF retentate powders indicated that, while lower in molecular mass than α -lactalbumin, it may be associated with the CN micelle and thus not be transmittable during MF. The band appeared fainter in MFR_{cold} powders (Fig. 2; lane 4) than in MFR_{warm} powders (Fig. 2; lane 3), indicating that proteolysis may have been more prevalent at 50 °C than at 8.9 °C. Hurt et al. (2010) found the presence of a similar band in SDS-PAGE gels of MF retentate streams after filtration of skim milk at 50 °C using 0.1 μ m pore sized UTP ceramic membranes. Jost, Brandsma, and Rizvi (1999) identified the band as proteose peptone component 5 (β -casein 1–105/1–107; Andrews, 1978) and suggested that the phosphoprotein remains associated with the casein micelle during MF and therefore does not permeate 0.1 μ m MF membranes even though its molecular mass is much less.

4. Conclusion

Cold MF and DF facilitated the formulation of a protein base with a casein profile close to human milk using a skim milk feed. Using this process it was demonstrated that two major formulation targets, a CN:WP ratio (40:60) and CN profile (β -CN), can be achieved. Other potential benefits of the cold MF and DF process were also identified, ranging from a reduction in β -lactoglobulin levels and reduced in-process proteolysis (lower levels of casein hydrolysis products). The manufacture of suitable infant formula protein base ingredients using integrated membrane systems will be an important development in next-generation IMF processing. Future work could also focus on the re-micellisation of β -CN through addition of κ -casein, to more closely mimic the protein profile of human milk while increasing heat stability.

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1 **Figure legends**

2

3 **Fig. 1.** Membrane filtration time of microfiltered diluted skim milk, measured as (A) a
4 function of permeate flux ($\text{L m}^{-2} \text{h}^{-1}$) and (B) total solids at 50 °C (□) and 8.9 °C (■).

5

6 **Fig. 2.** Reducing SDS-PAGE patterns (4–20% acrylamide gel) of retentate powders
7 from microfiltered and ultrafiltered skim milk. Lane 1, UF retentate produced after
8 microfiltration at 50 °C (UFR_{warm}); lane 2, UF retentate produced after microfiltration
9 at 8.9 °C (UFR_{cold}); lane 3, MF retentate produced by microfiltration at 50 °C
10 (MFR_{warm}); lane 4, MF retentate produced by microfiltration at 8.9 °C (MFR_{cold}); lane
11 5, α -lactalbumin standard; lane 6, β -lactoglobulin standard; lane 7, lactoferrin
12 standard.

13

14 **Fig. 3.** Reversed-phase HPLC profiles of (A) skim milk, (B) microfiltration retentate
15 at 50 °C (MFR_{warm}), (C) microfiltration retentate at 8.9 °C (MFR_{cold}), (D) UF retentate
16 produced after microfiltration at 8.9 °C (UFR_{cold}) and (E) UF retentate produced after
17 microfiltration at 50 °C (UFR_{warm}); eluate was measured at 214 nm.

18

Table 1Composition of microfiltration and ultrafiltration retentates after processing at 50 or 8.9 °C.^a

Parameter	Skim milk	MFR _{warm}	MFR _{cold}	UFR _{warm}	UFR _{cold}
Protein (% , w/w, dry basis)					
TPN	37.8 ± 2.1	74.3 ± 0.8	72.1 ± 2.2	44.2 ± 1.0	34.4 ± 2.2
NPN	0.03 ± 0.0	0.10 ± 0.0	0.01 ± 0.0	0.03 ± 0.0	0.03 ± 0.0
Fat (% , w/w, dry basis)	0.01 ± 0.0	0.81 ± 0.9	0.80 ± 0.6	0.40 ± 0.1	0.24 ± 0.2
Moisture content (% , w/w)		4.37 ± 0.8	4.69 ± 0.8	2.58 ± 0.5	2.21 ± 0.7
pH	6.64 ± 0.05	7.30 ± 0.1	7.22 ± 0.1	7.06 ± 0.0	7.08 ± 0.1

^a Abbreviations are: MFR_{warm}, retentate of microfiltration performed at 50 °C; MFR_{cold}, retentate of microfiltration performed at 8.9 °C; UFR_{warm}, ultrafiltration retentate after warm (50 °C) microfiltration; UFR_{cold}, ultrafiltration retentate after cold (8.9 °C) microfiltration; TPN, true protein nitrogen; NPN, non-protein nitrogen.

Table 2Protein profile of ultrafiltered retentate powders after processing at 50 or 8.9 °C. ^a

Protein	Skim milk	UFR _{warm}	UFR _{cold}
Total casein (% , w/w, of TP)	85.9 ^a	8.81 ^b	35.5 ^c
α _S -Casein (% , w/w, of TC)	50.1 ^a	0.00 ^b	0.00 ^c
β -Casein (% , w/w, of TC)	39.6 ^a	8.81 ^b	35.5 ^c
κ -Casein (% , w/w, of TC)	10.3 ^a	0.00 ^b	0.00 ^c
Total whey protein (% , w/w, of TP)	15.6 ^a	91.2 ^b	64.5 ^c
α -La (% , w/w, of TW)	17.7 ^a	19.1 ^b	17.9 ^c
β -Lg (% , w/w, TW)	82.3 ^a	72.0 ^b	46.6 ^c

^a Abbreviations are: UFR_{warm}, ultrafiltration retentate after warm (50 °C) microfiltration; UFR_{cold}, ultrafiltration retentate after cold (8.9 °C) microfiltration; α -La, α -lactalbumin; β -Lg, β -lactoglobulin; TP, total protein; TC, total casein; TW, total whey protein. Values are the means of 3 replicates \pm standard deviations; values within a row not sharing a common superscript letter differ significantly, $P < 0.05$.

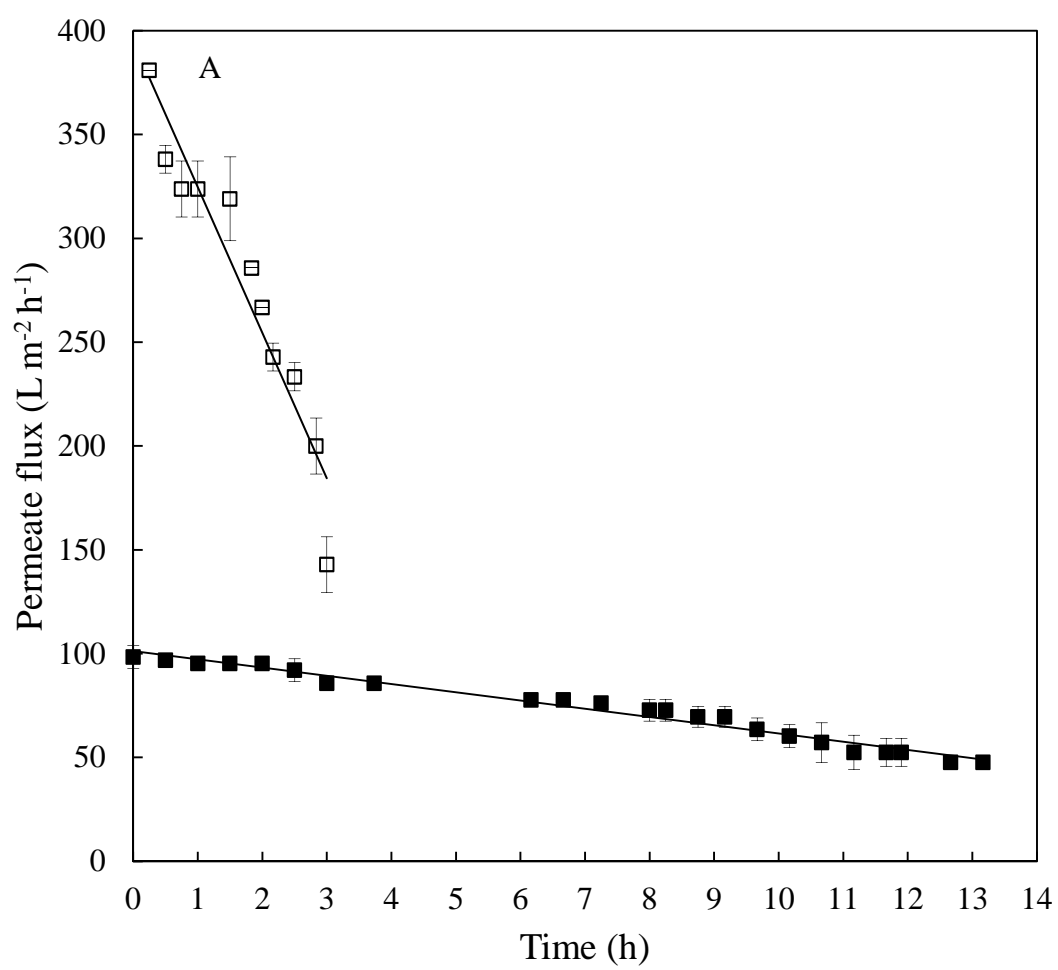
Table 3

Mineral profile of microfiltration and ultrafiltration retentate powders obtained at 50 or 8.9 °C. ^a

Process	Calcium	Magnesium	Sodium	Phosphorous	Potassium
MFR _{warm}	35.6 ^a	1.65 ^a	2.22 ^a	24.2 ^a	10.3 ^a
UFR _{warm}	24.2 ^b	4.34 ^b	23.8 ^b	20.6 ^b	70.4 ^b
MFR _{cold}	30.9 ^c	1.81 ^a	2.84 ^a	22.7 ^a	12.2 ^a
UFR _{cold}	29.2 ^d	4.44 ^b	31.4 ^b	27.5 ^c	66.3 ^b

^a MFR_{warm}, microfiltration retentate performed at 50 °C; MFR_{cold}, microfiltration retentate performed at 8.9 °C; UFR_{warm}, ultrafiltration retentate after warm (50 °C) microfiltration; UFR_{cold}, ultrafiltration retentate after cold (8.9 °C) microfiltration. All ultrafiltration processing was performed at 50 °C. Values (mg g⁻¹ of protein) are the means of 3 replicates ± standard deviations; values within a column not sharing a common superscript letter differ significantly, $P < 0.05$.

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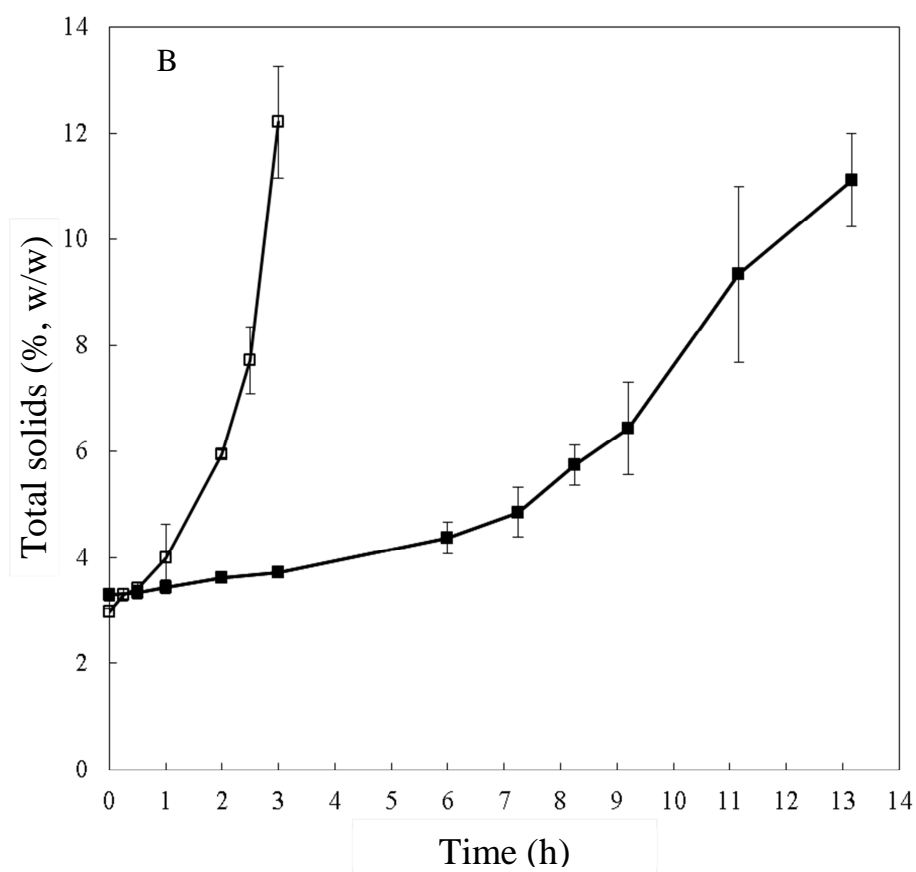


Fig. 1.

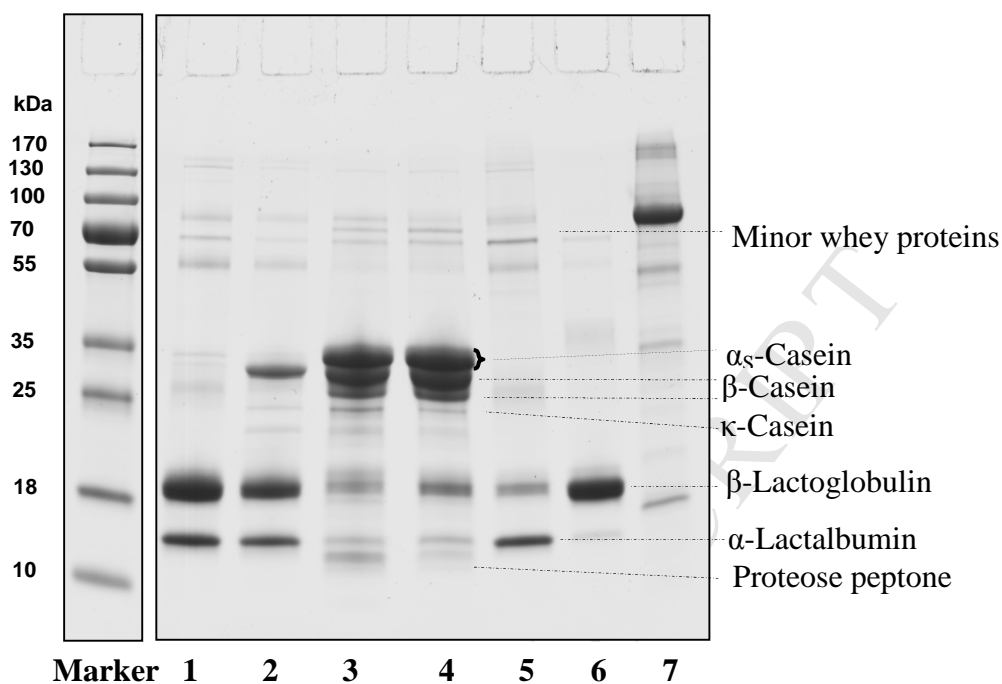
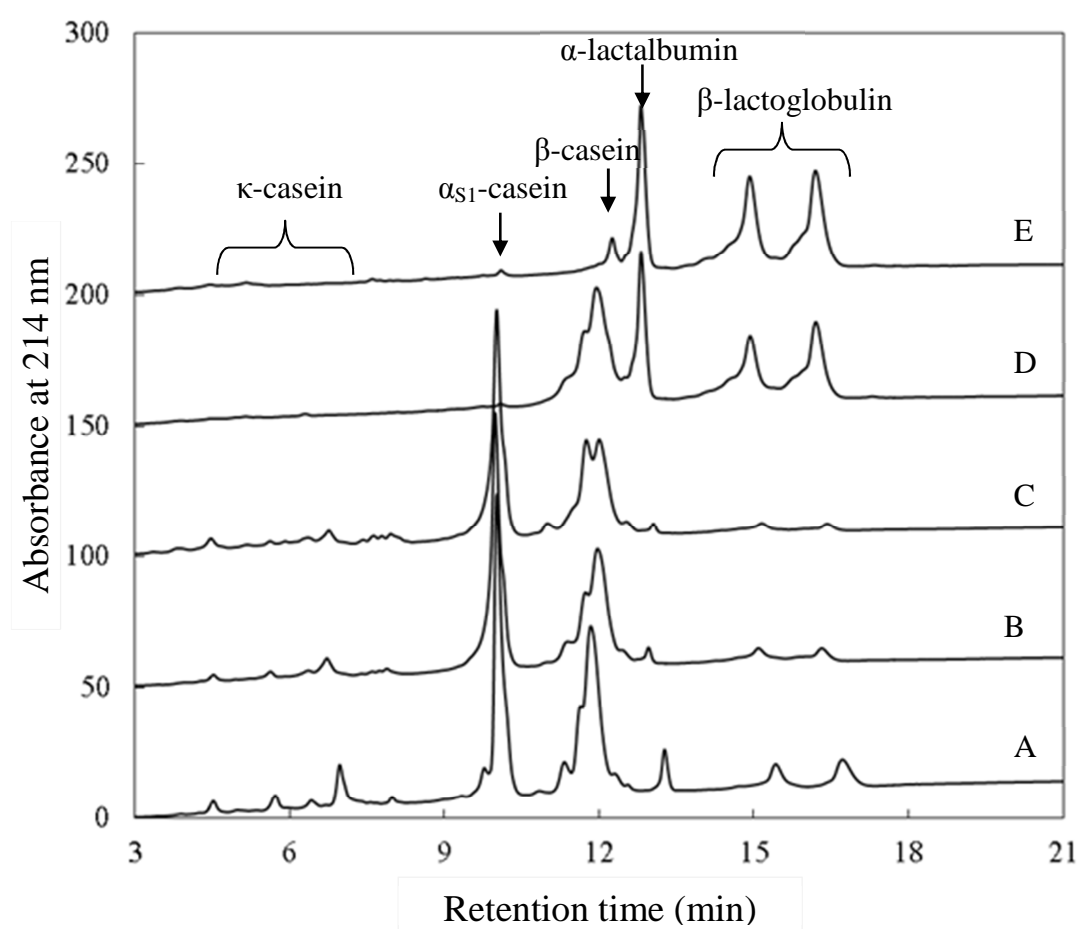


Fig. 2

67

**Fig. 3.**